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Commercial and nutraceutical quality of grafted melon cultivated under hydric stress

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Abstract: Water stress decreases the quality of fruit by generating reactive oxygen species. Grafting is a technique that can improve the efficiency of crop water usage. This work was performed in order to assess the effect of different water stresses on the commercial and nutraceutical quality of a melon fruit. Cantaloupe melon plants, grown under shade houses were grafted onto a creole pumpkin rootstock and grown with different water stresses (20, 30, and 40 kPa). The grafted melon plants under 30-kPa water stress (G30) showed greater fruit firmness and increased catalase activity. The G30 fruits showed an increase in GPX activity of up to 80% over the non-grafted plants. The GSH was higher in fruits subjected to the 40-kPa water tension. The superoxide dismutase showed a 15% greater inhibition in the fruits from the non-grafted plants. At higher water tensions, the DPPH antioxidant activity decreased, while the vitamin C content increased.

Keywords: antioxidants; *Cucumis melo*; water stress; nutraceuticals

Plants in the fields are always subject to a variety of changing environmental conditions (Mo et al. 2016). These can trigger various alterations within the plant, ranging from gene expression and cellular metabolism to changes in the plant's growth rate and productivity. Soil salinity, high solar radiation, nutrient deficits, low temperatures, and water stresses can be found among these changing conditions (Shao et al. 2008). Water stress, in particular, can have a great impact on the agricultural yield and productivity (Ding et al. 2017), as it is an important determinant in a plant's growth (Shao et al. 2008) and development given that water participates in various metabolic and physiological functions within the plant such as photosynthesis—and in the hemicellulose, pectin, and lignin content (Barbosa et al. 2014; Gall et al. 2015).

Mexico is a country considered with low water availability, thus, encouraging one to not only promote the efficiency of the resource in agriculture, but also to promote its use in a sustainable way (Salazar Moreno et al. 2014), therefore, water is one of the concerns of the Mexican people, becoming a matter of national security (Rolland, Vega Cárdenas 2010), since, in addition to in agricultural activities, the use of pesticides is one of the most common practices and that put the soils and waters at risk, where they reach other areas by the runoff, infiltration and erosion of the soils in areas where they have been used (Hernández, Hansen 2011).

The lack of water negatively affects processes such as the rate of photosynthesis, CO₂ fixation, and a decrease in the ribulose-1,5-bisphosphate carboxylase

activity leading to decreased cellular energy and increased production of reactive oxygen species (ROS). Plants counteract these ROS with diverse defence mechanisms including the production and subsequent activity of enzymatic and non-enzymatic antioxidants (Asada 2006), such as superoxide dismutase (SOD), peroxidases (POD), catalase (CAT), and ascorbate peroxidase (APX), among others (Wang et al. 2009), as well as betaine and proline compounds (Barzegar et al. 2017). These antioxidant compounds keep the concentration of ROS at low levels by reacting with the different active forms of oxygen, thanks to their ability to donate electrons (Gill, Tuteja 2010). The increased catalase, ascorbate peroxidase, and guaiacol peroxidase activity and malondialdehyde content have been seen in watermelons (*Citrullus lanatus* Thunb) cultivated under conditions of water stress (Yoosefzadeh Najafabadi et al. 2018). Other works have also shown that catalase activity and proline concentration increase in plants grown in water-scarce conditions, compared to those in which water is readily available (Barzegar et al. 2017). In recent years, the amount of nutraceutical compounds has been found to increase under conditions of controlled stress during the development stage of a crop (Vargas-Hernandez et al. 2017). Regulated deficit irrigation has also been seen to improve the quality of fruit in greenhouse crops by carefully supplying the appropriate amount of water (Yang et al. 2017). One report saw the fruit's vitamin C content increase by 55% over values obtained from field-grown fruits (Wang et al. 2017).

There have been some alternative, agricultural techniques developed for more efficient water management in crops. One such technique is grafting, a technique where the root system of a plant known to have resistance to some biotic or abiotic stress is used as a rootstock, while the aerial part of a commercial crop comprises the scion. Species of the *Cucurbitaceae* family are widely used as rootstocks for this technique (Gaion et al. 2018), as they favour root development and a subsequent increase in the crop productivity due to their efficiency and effectiveness in absorbing water and nutrients (Soteriou et al. 2017). A greater tolerance to abiotic stress, as well as the greater commercial and nutraceutical quality of the fruit (Mudge et al. 2009), are other benefit of using this technique, thanks to the increased adaptability of the plants under stress conditions. The translocation of molecules transmitted through the phloem (including proteins and nucleic

acids) has also been documented in grafted plants (Turnbull 2010). Sánchez et al. (Sánchez et al. 2016) reported that grafted tomato fruits exhibited a lower ROS generation accompanied by an increased catalase and superoxide dismutase activity, thus leading to an increase in the nutraceutical value. That finding falls in line with the importance (Rouphael et al. 2010) of the attributes in the search for rootstocks that improve fruit quality.

Melon cultivation is of great economic importance in Mexico. Its production occurs predominantly under open field conditions that leave it susceptible to water stress. The Lagunera region is one of the main melon producing areas (Ramírez et al. 2015) even though the edaphoclimatic characteristics of this area lead to adverse effects caused by water stress in plants, affecting the yield of the crop and the nutritional value of the fruit (Zeinalipour et al. 2017). Reductions in the parameters, such as fruit firmness, total soluble solids, and titratable acid (Barzegar et al. 2017) are caused by the morphological changes, such as the root length reduction (Yoosefzadeh Najafabadi et al. 2018), which leads to a concomitant reduction in the water and nutrient uptake (Gaytan Mascorro, Chew Madinaveitia 2014). An increase in the antioxidant defence, that is to say, the presence and activity of antioxidant enzymes in the plant stem and root accompanies these effects (Wang et al. 2009). Given all of the above, the purpose of the present work is to assess the effect of water stress on the commercial and nutraceutical quality of grafted melon cultivation.

MATERIALS AND METHODS

Location of experiment. This research was carried out under shade house conditions (30%) within the facilities of the Antonio Narro Autonomous Agrarian University, Coahuila de Zaragoza, Mexico between February to September 2017. The average temperature ranged from a minimum of 15 °C to a maximum of 28 °C, with an average relative humidity of 53% and photosynthetically active radiation ranging between 204.5 and 624.3 $\mu\text{mol}/\text{m}^2\cdot\text{s}$ between May to September 2017. The experiment was undertaken in a crop cycle for one year.

Plant material, growth and grafting conditions. Cantaloupe var. E25F.001 F1 (EnzaZaden) melon seedlings were used for the scions, while the rootstock used was the creole pumpkin *Cucurbita maxima* Ferro R2 (RijkZwaan). The creole pumpkin seeds

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Table 1. The physico-chemical properties of the soil and water used in the experiment

Variables	Water	Minerals
MO	–	1.22
pH	7.94	8.09
EC	0.97	–
N-NO ₃ ⁻	3.92	54.2
P-Olsen	–	21.7
K	2.73	898
Ca	77.6	5 387
Mg	22.3	477
Na ⁺	90.9	314
S-SO ₄	94.1	12.7
HCO ₃	309	17.10
CO ₃	0	–
Cl	87.5	–
Zn	0.0025	0.56
Mn	0.0007	7.16
Cu	0.0033	0.91
B	0.51	1.55
Fe	0.0059	5.26

MO – organic matter content (%); EC – electrical conductivity (dS/m); minerals expressed in ppm

were sown in a 60-cell polystyrene tray filled with Sunshine[®] Blend #3 peat moss. Seven days after the pumpkin seed sowing, the melon seeds were similarly sown. The planting of the variety and rootstock was conducted in a house with a 30% shade cloth.

The graft was carried out 12 days after transplanting when the seedlings had reached a stem diameter of 5 mm in the greenhouse conditions. The technique of the grafting approach can be found in (Oda 1995). The grafted seedlings were kept in greenhouse conditions with constant water misting to prevent tissue dehydration. Ten days after the grafting was carried out, the seedlings were transplanted to black, 12 L polyethylene containers containing loamy-sandy-clay soil (Table 1) with a saturation point of 48.40%, a field capacity of 25.80%, a withering point of 15.40%, and apparent density of 1.04 g/cm³ obtained from Ejido Petronilas, a melon-producing open field area. The soil was saturated with 3.2 L of water, previously characterised (Table 1), during the 24 hours before transplantation.

Irrigation following the transplant was planned according to the water tension readings from the tensiometers placed in three containers. After 24 hours, it was noted that 1 L of water reduced the water

tension in the soil by 50 kPa. Based on this figure, the water supply per container required to achieve three different water stresses (20, 30 and 40 kPa) was calculated. The plants were fertilised with a Steiner nutrient solution (Steiner 1961) according to the phenological stage while also maintaining the soil water tensions indicated above.

Treatments. The grafting state (grafted or non-grafted) and the three different water tensions (20, 30, and 40 kPa) were the experimental factors considered. The treatments consisted of a combination of grafted and non-grafted melon plants grown under different water stresses for a total of six treatments.

Quantification of commercial quality. The commercial quality was determined in the fruits obtained 125 days after grafting. The firmness of the fruit was determined with a penetrometer (Wagner Force Dual FDK 160). Briefly, the epicarp was removed, and then the fruit pulp was penetrated. Readings were taken in lb × 2 ozf. The quantification of the fruit's total soluble solids was performed with the help of a refractometer (Hanna, model HI 96801) by placing a drop of the pulp juice on the reader and taking readings, with units expressed in °Brix.

Quantification of nutraceutical quality. At 125 days after grafting, the fruit was harvested (once the net had formed and the apical part of the fruit demonstrated a slight softening) and frozen at 4 °C to halt respiration. The mesocarp was then selected and stored at – 86 °C in an ultra-freezer (VIP[®] Series SANYO) for 30–45 minutes. Finally, the tissue was lyophilised for 24 hours in a freeze-dryer (FreeZone 2.5 Liter Benchtop Freeze Dry System, Labconco).

Biomolecule extraction. The freeze-dried tissue was powdered, and 200 mg of the sample was placed in an Eppendorf tube along with 20 mg of polyvinylpyrrolidone (PVP) (Sigma-Aldrich). Subsequently, a 1.5 mL phosphate buffer 0.1 M (pH 7.0–7.2) was added. The sample was sonicated (Ultrasonic Cleaner Branson 1510) for 5 minutes and then centrifuged (Microcentrifuge Refrigerated Labnet Prism[™] R) at 12 500 revolutions per minute (rpm) for 10 min at 4 °C. The supernatant was decanted and filtered through a 0.45 µm pore size PVDF (polyvinyl difluoride) syringe filters (Ramos et al. 2010). The filtered residue was diluted in a 1 : 15 ratio with the phosphate buffer and stored in Eppendorf tubes at –86 °C.

Total proteins. For the quantification of the total proteins, 1 mL of the Bradford protein reagent

(Coomassie Brilliant Blue G-250) was added to 100 μ L of the biomolecule extract, mixed, and left to react for 2 minutes. Afterward, the absorbance at 595 nm was read with a spectrophotometer, the total proteins were determined by plotting the absorbances along the Bovine serum albumin calibration curve. The total protein was expressed in mg/g dry weight (Bradford 1976).

Catalase activity, CAT (EQ 1.11.1.6). Two reaction times, time 0 (T0) and time 1 (T1), were measured for the catalase activity determination. The blank reaction mixture consisted of 0.1 mL of the biomolecule extract, 1 mL of the phosphate buffer (pH 7.2), and 0.4 mL of H_2SO_4 (5%). The mixture for T0 was prepared by adding 0.1 mL of the biomolecule extract to 1 mL of H_2O_2 100 mM, and immediately after, 0.5 mL of H_2SO_4 (5%). The T1 reaction mixture was prepared in the same way except that the 0.5 mL of H_2SO_4 (5%) was applied after 1 min of reaction between the biomolecule extract and the H_2O_2 . The reaction was carried out at 20 °C with constant agitation. Finally, the consumption of H_2O_2 at 270 nm was measured in a spectrophotometer (Thermo Scientific Genesys 10S UV-Vis). The enzymatic activity was determined by plotting the absorbances along the H_2O_2 calibration curve. The catalase activity was expressed in mM of H_2O_2 /g·min (Cansev et al. 2011).

Glutathione peroxidase, GPX (EQ 1.11.1.9). The determination of the GPX activity was performed using the method established by Xue et al. (Xue et al. 2001) with H_2O_2 as the substrate. A mixture of 0.2 mL of the biomolecule extract plus 0.4 mL of the reduced glutathione (0.1 M) and 0.2 mL of Na_2HPO_4 (0.06 M) was placed in a test tube. This mixture was pre-heated in a water bath (IKA® HB 10 basic) at 25 °C for 5 minutes. Subsequently, the catalytic reaction was initiated by adding 0.2 mL of H_2O_2 1.3 mM. After 10 min, 1 mL of 1% trichloroacetic acid was added to stop the reaction. This reaction mixture was put in an ice bath for 30 min, then centrifuged at 3 000 rpm for 10 minutes. Afterward, 0.48 mL of the supernatant was taken and placed in a test tube, where 2.2 mL of Na_2HPO_4 (0.32 M) and 0.32 mL of 1 mM 5,5'-dithiobis-(2-nitrobenzoic acid) dye (DTNB) solution were added. Readings were taken with a UV-VIS spectrophotometer at 412 nm. The GPX enzymatic activity was determined by plotting the absorbance along a reduced glutathione calibration curve. The units of the activity were expressed in mM glutathione (g/min).

Reduced glutathione (GSH). The activity of glutathione was determined using the technique of Xue et al. (Xue et al. 2001). Briefly, 480 μ L of the biomolecule extract and 2.2 mL of Na_2HPO_4 0.32 M were placed in a test tube, shaken, 320 μ L of 1mM DTNB was added, and the mixture was shaken again. Readings were taken with a UV-VIS spectrophotometer at 412 nm. The GSH enzymatic activity was determined by plotting the absorbance along the calibration curve of the reduced glutathione.

Superoxide dismutase, SOD (EQ 1.15.1.1). The superoxide dismutase activity was determined using a SOD Assay Kit (Sigma-Aldrich® 19160) using a 96-well microplate and read on an ELISA (enzyme-linked immunosorbent assay) plate reader (BioTek ELx808) at 450 nm. The SOD activity was expressed as the % inhibition rate, derived from the equation described in the commercial kit.

DPPH (2,2-Diphenyl-1-picrylhydrazyl). The DPPH activity was determined using the commercial Antioxidant Assay Kit (Sigma-Aldrich® CS0790) and read on an ELISA plate reader (BioTek ELx808) at 405 nm. The antioxidant activity was determined by plotting the absorbances along a Trolox concentration standard curve and expressed as the DPPH antioxidant capacity (mMTrolox EQ 100 /g dry weight).

Vitamin C. Twenty grams of the sample were weighed out and placed in a mortar, where 10 mL of 2% HCl was added, and the sample was carefully ground to a homogeneous consistency. Afterward, 100 mL of distilled water was added. The sample was homogenised and then filtered through gauze. Three 10 mL aliquots of the filtrate were taken and titrated with Thielman's reagent. The vitamin C content was determined from the volume of the reagent spent. The resulting values were expressed in mg 100/g fresh weight (AOC 1995).

Experimental design. The treatments were evaluated according to a 2 × 3 factorial array and set up in a completely randomised block design. The first factor consisted of the grafted or non-grafted plants (G, NG), while the second factor considered the different water stresses (20, 30, and 40). Ten repetitions were used per treatment; the experimental unit consisted of one plant per container.

Statistical analysis. The data obtained from the fruit production and enzymatic activity were analysed using a one-way ANOVA (analysis of variance) and a comparison of means using Fisher's Least Significant Difference test (LSD, $P \leq 0.05$) with the InfoStat software (Ver. 2017).

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RESULTS AND DISCUSSION

The use of a rootstock is a technique that can help improve the water usage since the root system of the rootstock has a better and more vigorous development, is more efficient in its water use, and is more tolerant to water stresses (Kumar et al. 2017; Pradhan et al. 2017).

Commercial quality. Of the commercially important parameters considered, only the fruit firmness showed any statistical differences ($P \leq 0.05$) between the factors (Figure 1) and within the interactions (Table 2). Greater firmness was obtained in the fruits from the plants cultivated under 20 kPa water stress, while at higher tension (40 kPa), the firmness decreased by about 21%. The G30 interaction showed greater fruit firmness compared to the other grafted plants (G20 and G40) and exceeded the values recorded for the non-grafted plants at 30 and 40 kPa water tensions (NG30, NG40).

According to Sensoy et al. (Sensoy et al. 2007) and Barzegar et al. (Barzegar et al. 2017), the yields and commercial quality of melon fruit grown under open field conditions are tightly related to the irrigation regimes as well as the genotypes used. The results observed in Table 2 suggest that the different water stresses to which the melon plants (grafted and not grafted) were subjected did not significantly affect the content of the soluble solids, which are mainly the sugars in the fruit pulp. The high °Brix values recorded are above the USDA grade standards for the melon, where 9 °Brix is considered “good internal quality” and 11 °Brix as “very good internal quality” (USDA 2008). These high values can lead to a longer shelf life as the sugars

Table 2. Comparison of the commercial, qualitative variables of the melon fruit obtained by grafting and cultivated under the different water stresses

Treatments	Firmness (lb × 2 ozf)	TSS (°Brix)
G20	13.25 ^c	14.53 ^a
Interactions between grafting factor and hidric stress (kPa)	G30 21.33 ^a	14.60 ^a
	G40 13.73 ^c	15.83 ^a
	NG20 19.38 ^{ab}	14.65 ^a
	NG30 15.45 ^{bc}	14.73 ^a
	NG40 15.08 ^c	15.33 ^a
Coefficient of variation	17.25	7.36

† The means with different letters are significantly different (LSD, $\alpha \leq 0.05$)

stored in the fruit can be used during the respiration process.

Since fruit firmness is directly related to the calcium pectate in the cell wall (Salisbury, Ross 1994), the reduction of this parameter in the fruits obtained from plants grown at 40 kPa may be due to a reduction in the absorption and transfer of the calcium caused by the low water supply in the soil. The increase in the firmness of the fruits from the grafted plants cultivated at 30 kPa water tension may be related to the fact that the *Cucurbita* rootstock probably develops a root system than those of the non-grafted plants, leading to an improvement in the calcium absorption under conditions of high water tension, *i.e.*, a more efficient water and nutrient uptake (Kyriacou et al. 2017, 2018; Pradhan et al. 2017). In accordance with (Liu et al. 2010), the total soluble solids and fruit firmness are extensively subject to

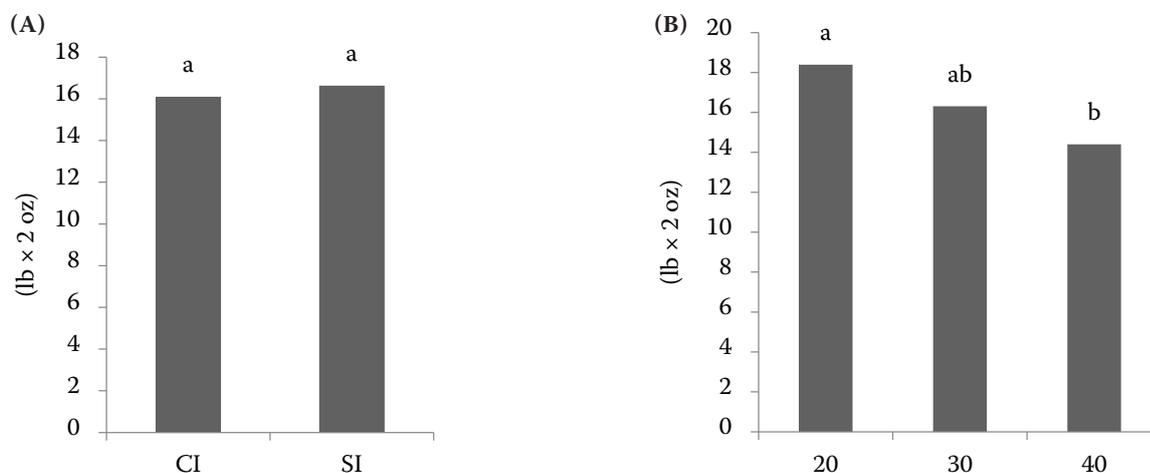


Figure 1. The effect of grafting (A) and water tension (B) on the melon fruit firmness

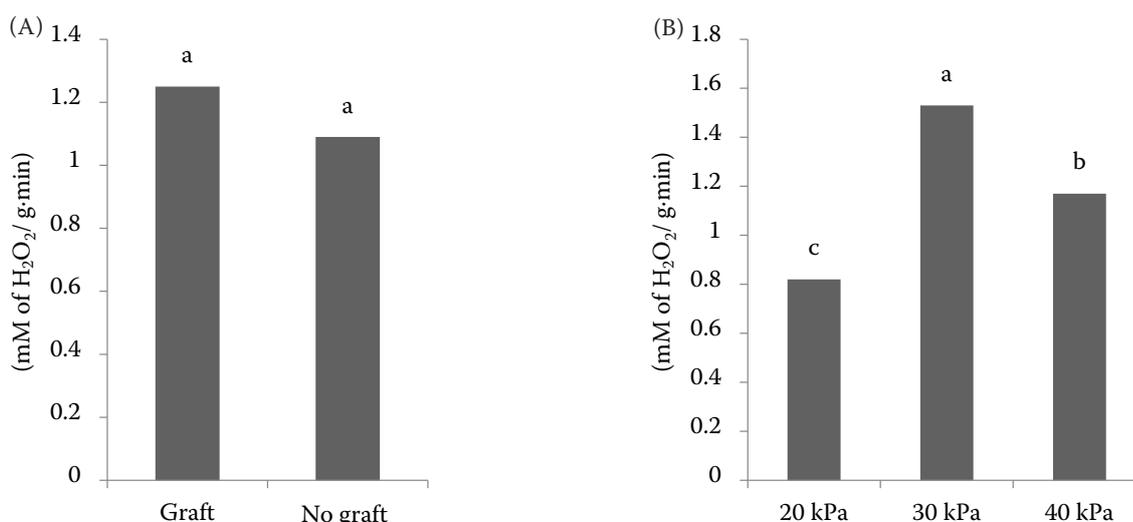


Figure 2. The effect of grafting (A) and water tension (B) on the catalase enzymatic activity

the rootstock interaction, so an optimal scion-rootstock combination is of utmost importance.

Nutraceutical quality. The results of the enzymatic and antioxidant activity assays are shown in Table 3. Statistical differences ($P \leq 0.05$) were observed for at least one factor and between the interactions for all the variables. Water deficit stress in plants induces the generation and accumulation of ROS, mainly H₂O₂ and O₂⁻, in different cellular compartments causing lipid peroxidation of the membranes and cellular damage. Plants developed a series of chemical responses to combat oxidative damage, such as the generation of enzymatic and non-enzymatic antioxidants to dissipate and control excess ROS (Yoosefzadeh Najafabadi et al. 2018).

The enzymatic activity of CAT showed significant differences in response to the water stress factor,

so that the graft factor did not prominently figure (Figure 2). The CAT enzyme activity in the grafted plants subjected to the water stress also showed significant differences. The fruit from the grafted melon plants cultivated under a tension of 20 and 30 kPa demonstrated CAT activity increases of 87 and 120%, respectively, compared to those from the non-grafted plants at 40 kPa water tension.

Catalase eliminates H₂O₂ by catalysing it to H₂O and O₂, primarily in the mitochondria (Zhang et al. 2010), so its activity plays an important role in drought tolerance in melons by keeping the H₂O₂ levels low, which inhibits the production of free radicals, and prevents lipid peroxidation of the membrane (Barzegar et al. 2017). The results of this paper suggest that the increased CAT activity in the fruit of the melon plants (both grafted and non-

Table 3. Comparison of the mean nutraceutical variables evaluated in the melon obtained by grafting and cultivated under the different water stresses.

	Treatment	TP	CAT	GPX	GHS	SOD	CAox	VIT C
Interactions between grafting factor and hydric stress (kPa)	G20	9.00 ^{dt}	0.71 ^b	1.28 ^c	2.12 ^{bc}	79.95 ^b	8.62 ^a	10.19 ^d
	G30	7.73 ^e	1.49 ^a	2.05 ^a	1.30 ^d	79.11 ^b	8.49 ^c	26.01 ^a
	G40	16.22 ^a	1.56 ^a	2.11 ^a	3.27 ^a	81.41 ^b	8.62 ^a	21.48 ^b
	NG20	12.82 ^c	0.93 ^b	0.72 ^d	3.01 ^a	92.81 ^a	8.64 ^a	17.08 ^c
	NG30	14.36 ^b	1.56 ^a	1.11 ^c	2.24 ^b	87.82 ^a	8.56 ^b	19.04 ^c
	NG40	15.10 ^{ab}	0.78 ^b	1.48 ^b	1.61 ^{cd}	93.35 ^a	8.48 ^c	22.43 ^b
Coefficient of variation		6.28	23.34	8.29	17.51	4.53	0.30	6.96

[†] The means with different letters are significantly different (LSD, $\alpha \leq 0.05$); TP – total proteins, CAT – catalase (mM of H₂O₂/g·min); GPX – glutathione peroxidase (mM of GSH reduced EQ/g·min); GHS – reduced glutathione (mM EQ of GSH reduced/g); SOD – superoxide dismutase (% inhibition); CAox DPPH – antioxidant capacity by 2,2-diphenyl-1-picrilhidrazyl (mM of Trolox EQ 100/g dry weight); VIT C – vitamin C (mg 100/g fresh weight)

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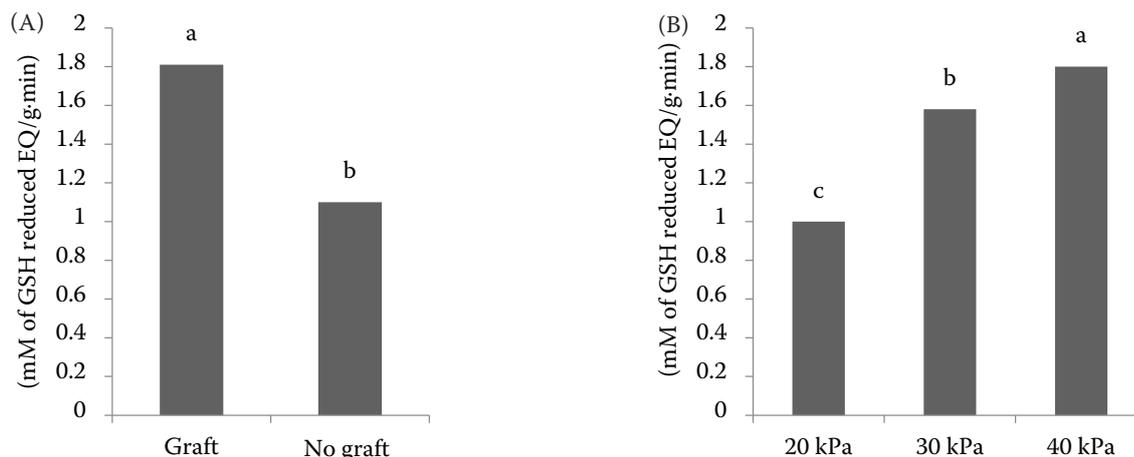


Figure 3. The effect of grafting (A) and water stress (B) on the GPX enzymatic activity

grafted) under the different water stress conditions could be related to the lower capacity for eliminating the ROS generated during the plant development under those conditions (Barbosa et al. 2017).

Regarding the GPX activity in the fruit, those from the grafted plants showed greater GPX activity (64%) compared to those from the non-grafted plants. On the other hand, increasing the water stress induces an increase in the GPX activity in the fruits by up to 80% at 40 kPa tension, compared to the 20 kPa tension (Figure 3). Considering the interactions, plants without grafting, in general, were found to possess less enzymatic activity in their fruit. The melon plants grafted on the pumpkin rootstock and cultivated under 30 and 40 kPa water tension showed the highest GPX enzyme activity values (60 and 65% greater, respectively), compared to those grown at 20 kPa tension. This enzyme is dependent on selenium and catalyses the reduction of H_2O_2 to H_2O through the glutathione-ascorbate cycle, using GSH as the reducing agent (Gill, Tuteja 2010; Labunskyy et al. 2014). Our results suggest that the fruits obtained from the

grafted plants under the high water stress show an increased GPX activity and, subsequently, a greater ability to eliminate H_2O_2 because of the water stress.

The differences between the factors for GSH, according to the difference of the means test, are presented (Figure 4), with the grafted plants presenting the largest quantity of GSH. A reduction in the GSH for the fruit from the plants under the 30 kPa water tension was observed. Regarding the interactions between the experimental factors, the grafted plants under 40 kPa tension (G40) resulted in a higher GSH content in the melon fruits, showing a slight increase over the plants without grafting. According to Gill and Tuteja (Gill, Tuteja 2010), among non-enzyme antioxidants, GSH shows a great ability to sequester ROS (1O_2 , H_2O_2 , and $OH\cdot$), making it a key player in the antioxidant defence. Zhang et al. (2010) reported a higher concentration of GSH in cucumber plant leaves grafted onto *Cucurbita ficifolia* under Cu stress. The same authors suggest that non-grafted plants present a higher concentration of ROS and a greater need for GSH to eliminate

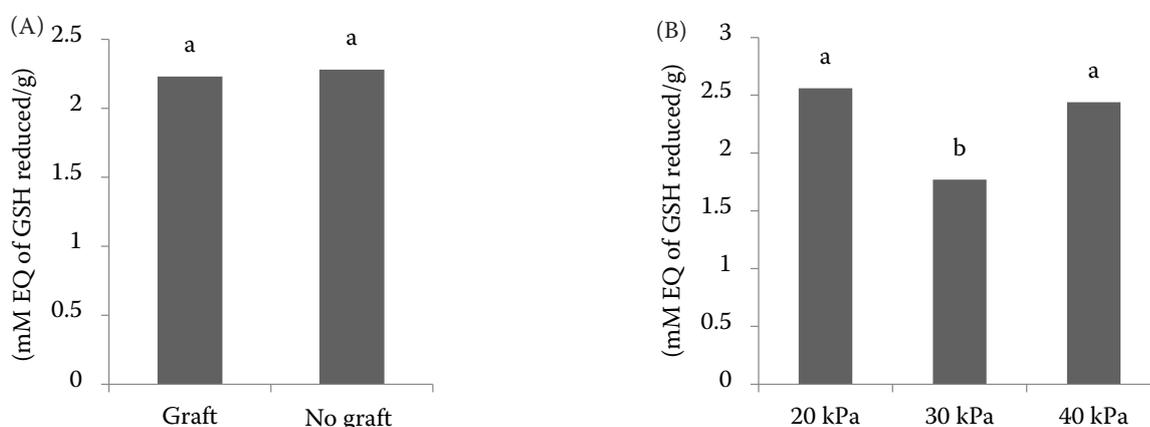


Figure 4. The difference in the effects of grafting (A) and water tension (B) on the GSH production

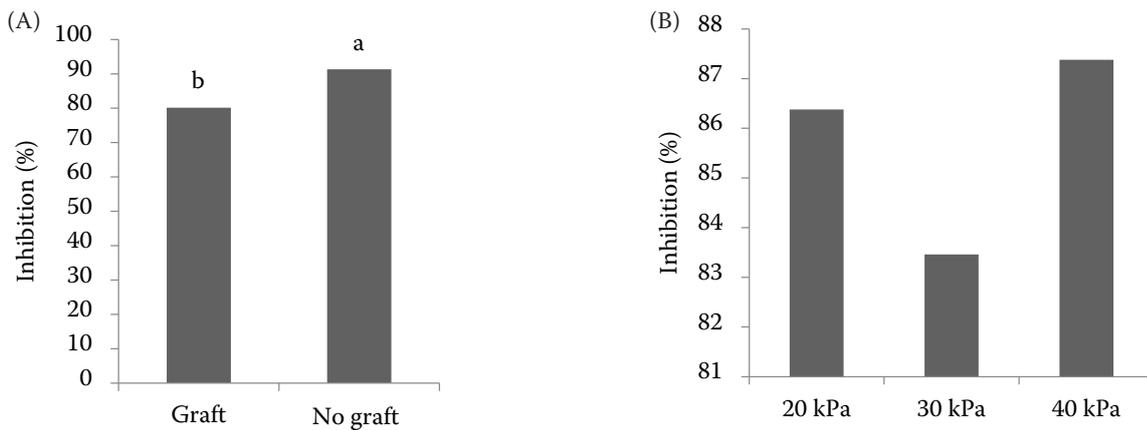


Figure 5. The effect of grafting (A) and water tension (B) on the percentage of the SOD inhibition

the effects of these radicals. Along the same line, the results obtained in this study suggest a similar response, as the fruits obtained from the grafted plants had a greater potential to capture and remove the ROS compared to the non-grafted plants under the high water stress conditions.

The SOD enzyme showed a higher percentage of inhibition in the non-grafted plants, 14% more than the grafted plants. There was no significant difference under the different water stresses (Figure 5). The non-grafted melon plants cultivated under the different water stresses showed 15% more SOD inhibition when compared to the grafted plants. The non-grafted, 40 kPa (NG40) treatment induced the highest SOD inhibition value, followed by the NG20 and NG30 treatments. In cellular compartments (chloroplast, mitochondria, cytoplasm, and peroxisome), the anion O_2^- , generated by the water stress, can be dismutated into H_2O_2 by SOD (Zhang et al. 2010). The higher percentage of the SOD inhibition

observed in the melon fruits from the non-grafted plants under the different water stresses could induce some degree of tolerance to the oxidative stress caused by the ROS, while there was a reduced SOD activity in the fruits obtained from the grafted plants. This result suggests that either grafting improved the tolerance to the different water stresses or that there was less ROS present in these fruits compared to those from the non-grafted plants.

The results of the DPPH antioxidant activity were similar for the grafted and non-grafted plants. The water stress factor indicated that the fruits of the melon plants under 20 kPa water tension exhibited an increased DPPH activity, but as the water tension increased, the activity decreased (Figure 6). Comparing the experimental treatments, NG30, NG40, and G30 presented with increased antioxidant activity. Barzegar et al. (2017) suggest that antioxidant activity in plants is promoted as a defence response to any alteration. However, the melon

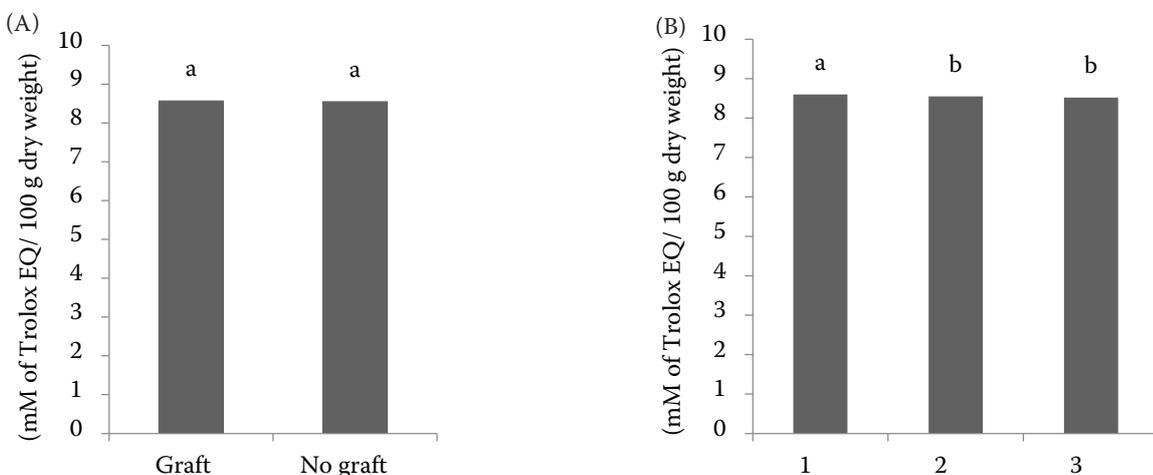


Figure 6. The influence of grafting (A) and water stress (B) on the DPPH antioxidant capacity of the melon fruit.

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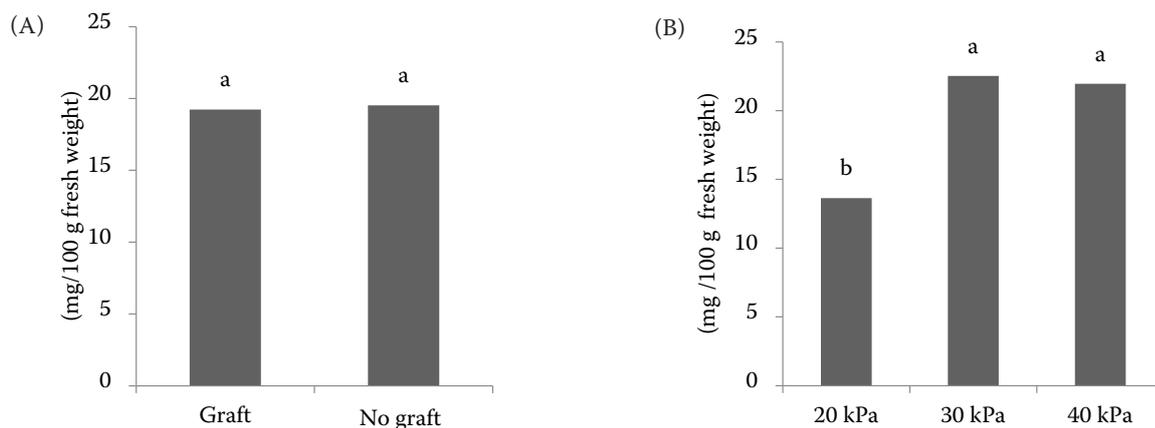


Figure 7. The vitamin C content of the melon fruit from the plants subjected to grafting (A) and water tension (B).

fruits from the plants (grafted or not grafted) under the water stress showed greater antioxidant activity at 20 kPa water tension, than when the water tension increased to 30 or 40 kPa. The greater antioxidant activity, as recorded by the DPPH assay, suggests an increased concentration of hydrophilic and lipophilic compounds.

Finally, the vitamin C content showed statistically similar averages when considering the graft factor, while increasing the water stress induced an increase in the fruit's vitamin C content up to 65% (Figure 7). Among the interactions, the lowest value was seen in the fruits from the grafted plants grown at the 20 kPa water tension, while the grafted plants cultivated at the 30 kPa water tension had a greater vitamin C content. This non-enzymatic, antioxidant compound, along with glutathione, has great importance for protecting the cell from oxidation. This compound helps sequester the ROS, such as $O_2^{\bullet-}$ and $OH\bullet$, by donating electrons within the glutathione-ascorbate cycle (Pignocchi, Foyer 2003; Gill, Tuteja 2010). High concentrations of this non-enzyme antioxidant would be expected under stress conditions, as was the case with the results obtained in the present study. An increase in the vitamin C content was seen in the fruits from the grafted plants subjected to a 30 kPa water stress, suggesting that the graft improved the fruit vitamin C synthesis.

CONCLUSION

Melon plants grafted and cultivated under water stress conditions yielded fruit with better firmness. The increased CAT and GPX activity suggested an antioxidant response in the melon fruits from the grafted plants, while the GSH content was high-

er in the fruits from the grafted plants under the 40 kPa stress. The SOD showed a higher percentage of plant inhibition in the non-grafted plants. The NG40 treatment possessed the highest SOD inhibition value. The DPPH antioxidant activity was not affected by grafting but responded to the conditions of the water stress. As the water tension increased, the DPPH activity decreased. The vitamin C content significantly increased due to increased water stress.

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